AN ANTIBIOTIC SUBSTANCE PRODUCED BY AN UNIDENTIFIED MEMBER OF THE GENUS STREPTOMYCES

BY

WALTER ANTHONY ZYGMUNT

B.S., University of Illinois, 1947
M.S., University of Illinois, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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IN THE GRADUATE COLLEGE OF THE
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AN ANTIBIOTIC SUBSTANCE PRODUCED BY AN UNIDENTIFIED MEMBER OF THE GENUS STREPTOMYCES

Fifty soil samples from various University of Illinois Experiment Stations located throughout Illinois were analyzed in this study. Seventy-five actinomycete cultures were isolated at random, and of these cultures, 41 (54.66%) displayed no antagonistic properties while 34 (45.33%) show antagonistic activity against one or more of the test organisms. *Micrococcus pyogenes* var. *aureus, Escherichia coli, Aerobacter aerogenes, Bacillus subtilis,* and*Sarcina lutea* comprised the group of test organisms.

One specific actinomycete, A412, because of marked antagonistic action against the genus Corynebacterium was selected for extensive study. The following lines of investigation were pursued: characterization of actinomycete A412, selection of a suitable liquid medium, extraction of a crude antibiotic substance, physical and chemical properties of the antibiotic, mode of action, in vitro studies, in vivo studies, and toxicity studies.

Screening of the soil samples:

A one gram portion of the soil in question was dispensed into a 99 ml. sterile, distilled water blank. Further dilution blanks were made and varied dilutions were plated on Conn's sodium asparaginate agar. Primary and secondary antibacterial spectra were determined by means of the cross-streak technique.

Cultivation in a liquid medium:

The following medium was employed for cultivation of

actinomycete A412:

Soybean meal	1.0 %
Dextrose	1.0 %
Sodium chloride	0.5 %
Curbay B. G. salts	0.05%
Calcium carbonate	0.1 %
Dry soil	1.0 %
Distilled water to volume	
Adjust to pH 7.0	

Extraction of the antibiotic substance:

Culture filtrates of actinomycete A412 were adsorbed on Darco G-60 and eluted with equal volumes of acetone. The eluates were allowed to evaporate to dryness at 55 C., and distilled water or physiological salt solution were employed as solvents for the acetone residue. The crude preparation was designated as preparation Z.

Properties of preparation Z and culture filtrates of actinomycete A 412:

(1) Thermostable in a neutral or an acid medium

(2) Relatively stable in the presence of whole horse blood or horse blood serum

(3) Non hemolytic to horse red blood cells

(4) Stable in the presence of flourescent lighting

(5) Stable in the presence of cysteine hydrochloride, stannous chloride, potassium periodate, and potassium permanganate.

(6) Stable in the presence of pancreatin and trypsin

Mode of action:

Preparation Z or actively growing cultures of actinomycete A412 failed to display complete bacteriolytic action against

viable or heat killed cells of preparation Z susceptible test organisms. Experiments were devised to demonstrate the fact that preparation Z was bacteriostatic and bactericidal in its mode of action.

In vitro studies:

I. Bacterial spectrum:

The test medium was brain heart infustion broth (Bacto) in 10 ml. aliquots. Additional media were employed in cultivation of *Diplococcus pneumoniae, Mycobacterium tuberculosis* var. *hominis* ATCC 9360, and members of the genus Clostridium. One ml. aliquots of sterile, whole horse blood were added asceptically to tubes of brain heart infusion broth (Bacto) in cultivation of the pneumococcus. The medium for the anaerobes was Brewer's thioglycollate broth (Bacto). The following medium was used for cultivation of *Mycobacterium tuberculosis*: asparagine 5.0 grams, monopotassium phosphate 5.0 grams, magnesium citrate 2.5 grams, magnesium sulfate .7 H_20 0.5 gram, glycerine 20 ml., distilled water to make a liter - adjust to pH 7.0 with 40% NaOH.

The inoculum, with the exception of *Mycobacterium tuberculosis,* consisted of 0.1 ml. of an 18-24 hour, 37 C. culture of the test organism in the test medium. Tubes designated as experimental contained 2.0 milligrams of preparation Z in addition to the inoculum and test medium. Tubes containing the test medium and the inoculum but no preparation Z served as controls. All tubes were incubated for 24-48 hours at 37 C. and examined macroscopically for the presence or absence of growth.

II. Yeast spectrum:

The test medium was as follows: peptone (Bacto) 0.5%, yeast extract (Bacto) 0.3%, malt extract (Bacto) 0.3%, dextrose 1.0%, and distilled water to volume (adjust to pH 6.8). The inoculum was 0.1 ml. of a 48 hour, room temperature culture of the yeast culture into 10 ml. aliquots of the

above medium. Experimental tubes contained 10 milligrams of preparation Z in addition to the inoculum and test medium. Tubes serving as controls contained no preparation Z. Upon completion of a 72-96 hour incubation period at room temperature, the tubes were observed macroscopically for the presence or absence of growth.

III. Mold spectrum:

The above medium was also utilized for the mold study. All tubes, 10 ml. aliquots, of the test medium were inoculated with 0.1 ml. of a spore suspension of the mold in question. In addition the experimental tubes contained 20 milligrams of preparation Z. Control tubes contained no preparation Z. All tubes were incubated at room temperature and observed macroscopically for the degree of growth at the end of a 5 day incubation period.

IV. Actinomycete spectrum:

The test medium, Emerson's broth, was dispensed in 10 ml. aliquots. All tubes were inoculated with 0.1 ml. of a spore suspension of the test actinomycete. Control tubes contained no preparation Z, whereas experimental tubes contained 20 milligrams of preparation Z. Upon completion of a 3 day incubation period at 30 C., all tubes were examined macroscopically for the presence or absence of growth.

Microorganisms non susceptible to the inhibitive action of prep. Z:

Shigella paradysenteriae - Newcastle

Escherichia coli
Aerobacter aerogenes
Alcaligenes faecalis
Shigella sonnei
Shigella alkalescens
Proteus vulgaris
Salmonella typhosa
Rhodotorula sanguinea
Brettanomyces bruxellensis
Torula utilis
Candida pseudotropicalis
Debaromyces globosus
Kloeckera brevis
Mycoderma cerevisiae

Salmonella typhimurium
Salmonella pullorm
Samonella choleraesuis
Pasteurella pestis
Serratia marcescens
Brucella abortus
Brucella suis
Bricella melitensis
Pseudomonas aeruginosa
Klebsiella pneumoniae
Klebsiella capsulata
Vibrio comma
Saccharomyces cerevisiae
Saccharomyces carlsbergensis
Rhodotorula rosea
Pichia kluyveri
Schizosaccharomyces pombe
Zygosaccharomyces barkeri
Zygohansenula californicus
Zygopichia farinosa
Absidia coerulea
Rhizopus nigricans
Aspergillus niger
Cladosporium fulvum
Thamnidium elegans
Penicillium roqueforti
Mucor generensis
Fusarium moniliformis
Alternaria species

Microorganisms susceptible to the inhibitive action of prep. Z:

Micrococcus flavus
Micrococcus lysodeikticus
Micrococcus pyogenes var. *albus*
Micrococcus pyogenes var. *aureus*
Bacillus anthracis
Bacillus cereus
Bacillus mycoides
Bacillus polymyxa
Bacillus subtilis
Corynebacterium diphtheriae - gravis A578
Corynebacterium diphtheriae - intermedius
Corynebacterium diphtheriae - mitis
Corynebacterium renale
Mycobacterium smegmatis
Mycobacterium tuberculosis var. *hominis* ATCC 607
Mycobacterium tuberculosis var. *hominis* ATCC 9360 (H37Rv)
Streptococcus pyogenes
Streptococcus salivarius
Listeria monocytogenes
Sarcina lutea

Erysipelothrix rhusiopathiae
Diplococcus pneumoniae - type I
Diplococcus pneumoniae - type III
Lactobacillus casei
Clostridium sporogenes
Clostridium perfringens
Clostridium tetani
Clostridium botulinum - type A
Nocardia asteroides
Streptomyces griseus
Micromonospora species

In vivo studies:

Preparation Z was of chemotherapeutic value in protecting certain laboratory animals against diphtheria and pneumonia.

Toxicity studies:

Preparation Z was relatively non toxic with respect to gross toxicity in various laboratory animals.

Summary:

Actinomycete A412 an unidentified member of the genus Streptomyces produces an antibiotic substance, designated as preparation Z in a chemically crude state, antagonistic to certain gram positive bacteria and certain members of the Nocardia, Streptomyces, and Micromonospora genera.

On the basis of this study which included the following phases: characterization of actinomycete A412, selection of a suitable liquid medium, extraction of a crude antibiotic substance, physical and chemical properties of the antibiotic, mode of action, in vitro studies, in vivo studies, and toxicity studies, it would appear that preparation Z has chemotherapeutic potentialities.

VITA

Walter Anthony Zygmunt was born on March 24, 1924 in Calumet City, Illinois, where he attended elementary and secondary schools. Upon graduation from high school in 1941, he entered the University of Illinois. After completion of his sophomore year, he enlisted in the United States Army. He was assigned to the 316th Field Artillery Battalion of the 81st Infantry Division and saw action in the Pacific Theatre of Operations.

He resumed his studies at the University of Illinois in September 1946 and was awarded a B.S. degree in Bacteriology in September 1947. He received a M.S. degree in Bacteriology from the University of Illinois in August 1948.

Since September 1948, he has held a graduate assistantship in the Department of Bacteriology, and during this period he has engaged in research and instruction.

The author is a member of Phi Eta Sigma, Phi Beta Kappa, Sigma Xi, Society of American Bacteriologists, Society of Illinois Bacteriologists, and the American Association for the Advancement of Science.